



# Prevalence of serum antibodies to bovine herpesvirus-1 and bovine viral diarrhea virus in beef cattle in Uruguay

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## Abstract

Our objective was to determine the prevalence of serum antibodies to bovine herpesvirus-1 (BHV-1) and bovine viral diarrhea (BVD) virus in beef cattle in Uruguay. A random sample of 230 herds selected with probability proportional to population size based on the number of cattle was chosen from a list frame of all registered livestock farms as of June 1999. Sera from up to 10 heifers, cows and bulls (up to 30 sera total per herd) were collected on selected farms between March 2000 and March 2001 and evaluated by means of enzyme-linked immunosorbent assays (ELISAs). Overall, 6358 serum samples were evaluated. We also collected data on previous diagnosis of BHV-1 or BVD infections and on the use of vaccines against these agents.

The estimated prevalence of exposure to BHV-1 and BVD at the herd level for the Uruguayan beef population was 99% and 100%, respectively. Approximately 37% of beef cattle in Uruguay have been exposed to BHV-1 and 69% to BVD virus. Only 3% of beef herds in Uruguay regularly (typically, annually) use vaccines against either of these agents.

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## 1. Introduction

Profitability of beef cattle herds depends on cost control and production of saleable product. Infection with bovine herpesvirus-1 (BHV-1) or bovine viral diarrhoea (BVD) virus can decrease reproductive efficiency (Miller et al., 1991; Guarino, 2000; Inui et al., 2000).

The main route of transmission of BHV-1 is by direct contact between animals through nasal, ocular, and genital secretions from infected animals and also by infected semen. Latent infections are possible, making it difficult to control exposure of new animals by relying on clinical examination and/or quarantine. Clinical manifestations of BHV-1 infections relevant to the reproductive system can include abortions and vulvovaginitis (Miller et al., 1991; Van Oirschot, 1995; Guarino, 1997).

The BHV-1 has frequently been associated with clinical disease in Uruguay since it was first isolated in 1981 (Guarino et al., 1982). Though there have been some studies in dairy herds, no information has been available on the extent of exposure to the virus among beef cattle in Uruguay, making it difficult to make recommendations on appropriate control strategies or to estimate the potential impact of BHV-1 on overall reproductive efficiency (Guarino, 1997; Saizar, 1997, 1998; Mederos and Hirigoyen, 1998; Gil et al., 2000; Guarino et al., 2000; Nuñez et al., 2000).

Infection with BVD virus is recognized throughout the world as one of the main causes of reproductive disorders (Bolin and Ridpath, 1996). The BVD virus is usually transmitted between animals by inhalation or ingestion of nasal or ocular secretions, saliva, urine, or feces. The virus can also be transmitted venereally in semen from an infected bull, or by transfer of contaminated embryos. Exposure of the fetus can result in embryonic absorption, mummifications, abortions, congenital malformations, or the birth of apparently normal calves that are persistently infected and shed large amounts of BVD virus (Brownlie, 1985; Houe, 1995).

Though BVD virus infections have been suspected in Uruguay since the 1980s it was first demonstrated via immunohistochemistry techniques in 1996 (Saizar, 1998; Guarino, 2000). Without information on prevalence and distribution of BVD virus in the country it has been difficult to recommend appropriate control strategies and to assess the potential economic impact on beef cattle production in Uruguay.

Our objective was to estimate the national prevalence of exposure to BHV-1 and BVD virus in breeding beef cattle and to assess use of BHV-1 and BVD virus vaccination.

## 2. Materials and methods

### 2.1. Sampling

#### 2.1.1. Herds

Each year in Uruguay, all farms with livestock are required to register and declare the number of animals of various types that are present on the farms. Farms (herds) for this study were randomly selected with probability proportional to size based on the number of cattle using the 1999 registration data and using a computer-generated list. This project was conducted as part of a broader study to evaluate health and management on beef cattle herds. Based on field and laboratory resources available, an overall sample size of 230 farms (one herd each) was manageable and we subsequently calculated that an overall sample size of 230 herds would allow the estimation of a herd-level prevalence of 18% within 5% with 95% confidence.

### 2.1.2. Animals

In the second stage, animals were selected from among three animal classes: bulls ( $\geq 2$  years of age), breeding cows (have calved at least once,  $\geq 3$  years of age), and replacement heifers (weaned heifers that have not yet calved,  $< 3$  years of age). Based on available laboratory resources, up to 10 animals were sampled systematically within each category. We subsequently calculated that such a sample size would provide 95% confidence of detecting at least one infected animal if the within-herd prevalence for one of these three categories was at least 26%.

### 2.1.3. Serum samples

The blood samples were collected from the jugular or tail vein. The blood samples were sent to the central veterinary reference laboratory or regional laboratories where the samples were centrifuged and the serum was harvested and stored at  $-20^{\circ}\text{C}$  until it was processed. A total of 6358 sera from 230 herds were processed for detection of antibodies against BHV-1 and BVD virus using a commercially available ELISA (Svanovir, Svanova Biotech AB Uppsala Science Park SE-751 83 Uppsala, Sweden) following the manufacturer's protocol. The positive reaction was indicated by a change in color detected using a spectrometer at 450 nm. The animal-level sensitivity and specificity of the test for BHV-1 antibodies are 98% and 99% respectively whereas for BVD virus antibodies the animal-level sensitivity and specificity are 97.9% and 99.7%, respectively (Solis-Calderon et al., 2003, 2005).

## 2.2. Data collection

At the time of blood-sample collection, a questionnaire written in Spanish was administered in person to collect information on herd demographics and herd management. (The questionnaire, in Spanish, is available from the corresponding author.) All questionnaires were administered by one of nine people. Prior to the start of the study, those administering the questionnaires received training to assure consistent and complete recording of the data. Specifically, questions were directed at the regular (generally annual) use of vaccines and previous history of a clinical- or laboratory-based diagnosis of BHV-1 or BVD infections. Administration of the questionnaire took  $\sim 20$  min per herd.

## 2.3. Data analysis

We analyzed the data using software (Intercooled STATA version 7) to account for the study design. The data were weighted to account for the sampling probability and non-response (Dargatz and Hill, 1996). Estimates of the prevalence of exposure to BHV-1 and BVD viruses were made based on the animal class and vaccination status and compared using a Pearson chi-square statistic with Rao and Scott second-order correction to account for clustering of samples by herd (Rao and Scott, 1981). This procedure results in an  $F$  statistic. In all cases two-tailed tests were used. A  $P$ -value of  $< 0.05$  was considered significant. Herds were classified as positive if one or more animals tested positive. Animal-level apparent prevalences were adjusted based on the sensitivity and specificity of the serologic tests as described previously (Greiner and Gardner, 2000). Herd-level prevalences were not adjusted for sensitivity and specificity of the tests used.

## 3. Results

Overall, 6358 serum samples (2271 from heifers, 2285 from cows, and 1802 from bulls) were collected and evaluated. The number of samples per herd ranged from 9 to 30.

Table 1

Percent of beef cattle herds by within-herd prevalence of bovine herpes virus-1 and bovine viral diarrhea virus antibodies (230 beef cattle herds, Uruguay, 3/2000–3/2001)

Within-herd prevalence (%)	BHV-1		BVD	
	Percent of herds	95% CI	Percent of herds	95% CI
0	1	0, 4	0	–, –
1–25	45	32, 59	13	5, 30
26–50	35	23, 50	20	10, 37
51–75	15	9, 22	25	16, 36
76–100	4	2, 8	42	30, 55

The estimated national herd-level prevalence of exposure to BHV-1 was 99% while 100% of the herds had evidence of exposure to BVD virus. The animal-level prevalence for antibodies to BHV-1 was estimated at 37% (95% confidence interval (CI): 34, 40) and for BVD antibodies it was 69% (95% CI: 65, 73).

Overall, 45% of herds had one or more animals with antibodies to BHV-1 but a within-herd prevalence of  $\leq 25\%$  (Table 1).

The largest percentage of herds (42%) had a within-herd prevalence of BVD antibodies  $\geq 76\%$  (Table 1). Over 51% of the animals tested were positive on 67% of the herds.

The proportion of animals with antibodies to BHV-1 was significantly different ( $F = 161.43$ ; d.f. = 1.25, 286.45;  $P < 0.001$ ) by animal class. Heifers had the lowest prevalence (11%, 95% CI: 7, 14) compared to cows (44%, 95% CI: 40, 49), and bulls (88%, 95% CI: 84, 90). In over half (59%) of herds, all samples from the heifers were negative for BHV-1 antibodies. The prevalence of BHV-1 antibodies was different ( $F = 3.87$ ; d.f. = 2.70, 619.40;  $P = 0.01$ ) by herd size (Fig. 1). When the prevalence of BHV-1 antibodies was evaluated within-animal class by herd size, significant differences were detected for cows ( $F = 4.47$ ; d.f. = 2.56, 573.06;  $P = 0.01$ ) and bulls ( $F = 7.55$ ; d.f. = 2.60, 594.44;  $P < 0.001$ ) by herd size but not for heifers ( $F = 0.61$ ; d.f. = 2.59, 564.71;  $P = 0.58$ ). In each of these the prevalence was lowest in the smallest herd-size category (Table 2).

There were significant differences in the prevalence of BVD antibodies by animal class ( $F = 34.93$ ; d.f. = 1.40, 321.06;  $P < 0.001$ ). For heifers, 55% (95% CI: 50, 61) had antibodies to BVD compared to 73% (95% CI: 69, 76) of cows and 69% (95% CI: 63, 74) of bulls. The prevalence of BVD antibodies was different ( $F = 2.92$ ; d.f. = 2.77, 635.06;  $P = 0.04$ ) by herd size

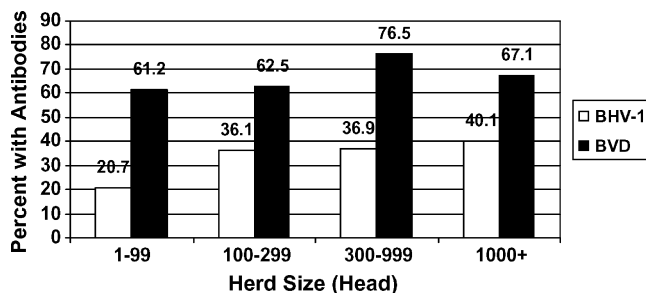


Fig. 1. Percent of animals with antibodies to bovine herpes virus-1 or bovine viral diarrhea viruses by herd size (230 beef cattle herds, Uruguay, 3/2000–3/2001).

Table 2  
Prevalence of bovine herpes virus-1 and bovine viral diarrhea virus antibodies by animal class and herd size (230 beef cattle herds, Uruguay, 3/2000–3/2001)

Herd size (total cattle, herd)	Heifers		Cows		Bulls	
	Prevalence (%)	95% CI	Prevalence (%)	95% CI	Prevalence (%)	95% CI
BHV-1						
1–99	4	0, 10	24	17, 30	69	46, 92
100–299	9	2, 16	43	32, 54	88	79, 97
300–999	12	5, 19	46	38, 54	82	76, 88
≥1000	11	7, 15	48	43, 54	96	93, 99
BVD						
1–99	34	16, 52	69	53, 84	65	39, 90
100–299	49	34, 65	67	58, 75	73	61, 84
300–999	63	54, 73	83	77, 88	78	71, 85
≥1000	56	48, 65	72	66, 78	66	57, 75

(Fig. 1). Prevalence of BVD antibodies by herd-size was not different for heifers ( $F = 2.44$ ; d.f. = 2.95, 648.73;  $P = 0.07$ ) and bulls ( $F = 1.38$ ; d.f. = 2.52, 574.03;  $P = 0.25$ ), but was a different ( $F = 2.98$ ; d.f. = 2.83, 632.86;  $P = 0.03$ ) for cows (Table 2).

An estimated 3% (95% CI: 1, 5) of producers regularly vaccinated for BHV-1 and/or BVD. For cattle on herds reporting vaccination the prevalence of antibodies to BHV-1 and BVD were 56% and 77%, respectively. Fewer ( $F = 13.61$ ; d.f. = 1.00, 229.00;  $P < 0.001$ ) animals (35%) were seropositive for BHV-1 in herds that did not report vaccination compared to those that reported regular vaccination. The prevalence of BVD antibodies in herds not reporting vaccination (67%) was not different when compared to herds that did vaccinate (77%) ( $F = 3.55$ ; d.f. = 1.00, 229.00,  $P = 0.06$ ).

Vaccination for BHV-1 was much more common ( $F = 76.37$ ; d.f. = 1.00, 228.00;  $P < 0.001$ ) among herds that had a previous diagnosis (clinical- or laboratory-based) of BHV-1 (Table 3) compared to herds without a previous diagnosis. Similarly, vaccination for BVD was much more common ( $F = 59.58$ ; d.f. = 1.00, 228.00;  $P < 0.001$ ) among herds that had a previous diagnosis (clinical- or laboratory-based) of BVD (Table 3).

4. Discussion

We found that beef cattle exposure to BHV-1 and BVD virus is common in Uruguay, comparable to those from other parts of the world. In a study of U.S. beef cattle herds, 90% of

Table 3  
Percent of herds with or without a previous diagnosis of bovine herpes virus-1 or bovine viral diarrhea virus infections that vaccinate with the respective antigen (230 beef cattle herds, Uruguay, 3/2000–3/2001)

Virus	Previous diagnosis	Vaccinate	
		Percent of herds	95% CI
BHV-1	Yes	57	23, 86
	No	1	1, 4
BVD	Yes	49	17, 82
	No	1	1, 3

herds had animals with antibodies to BVD virus and >68% of the animals tested had been exposed to BVD virus either through vaccination or naturally (Paisley et al., 1996). Approximately 54% of unvaccinated beef cattle in Mexico were seropositive to BHV-1 (Solis-Calderon et al., 2003). However, the estimated prevalence of BVD virus exposure among unvaccinated beef cattle in the Yucatan, Mexico was 14% (Solis-Calderon et al., 2005). In another study of randomly selected dairy and beef cattle in Mexico the animal-level prevalences for BVD antibodies were 71% and 63%, respectively (Suzan et al., 1983). In a study from Brazil, the herd- and animal-level prevalences of BVD antibodies were 90% and 56%, respectively (Canal et al., 1998). A study of beef cattle in Venezuela showed animal-level prevalences of 36% and 67% for BVD and BHV-1 antibodies, respectively (Obando et al., 1999). The herd- and animal-level prevalence for BVD antibodies was generally similar elsewhere in the world (Houe, 1999; Houe, 2005; Kapil and Basaraba, 1997; Lindberg and Houe, 2005; Boelaert et al., 2000).

That such a large percentage of the heifers were seronegative to BHV-1 is consistent with what others have seen and suggests that exposure to the agent is occurring at the time of breeding (Inui et al., 2000).

The within-herd prevalence of BVD antibodies was similar to that observed previously in dairy cattle in the county of Florida in Uruguay (Gil et al., 2000) with the largest proportion of herds having a prevalence of  $\geq 76\%$ . No herds were completely negative for BVD exposure. The similar prevalence of antibodies to BVD virus across classes of animals might indicate relatively early exposure to the BVD virus, perhaps through exposure to persistently infected calves.

Given the uncommon use of vaccine in beef herds in Uruguay (3% of producers regularly used vaccines with either BHV-1 or BVD antigens), most of the positive samples can be attributed to natural exposure to the agents. From this study it appears that both of these disease agents are widespread in the Uruguayan beef herd. Studies to quantify the impacts of these viruses on animal health and production are warranted. In addition, evaluation of the potential effectiveness of the vaccines containing these antigens that are currently available in Uruguay and other biosecurity measures in mitigating adverse effects on health and production should be undertaken.

## 5. Conclusion

Our results suggest that exposure to BHV-1 and BVD virus in Uruguayan beef cattle is common; the animal-level seroprevalences were 37% and 69%, respectively for samples collected in 2000–2001. Furthermore, given the limited use of vaccine in this population, most of this is attributed to natural exposure.

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